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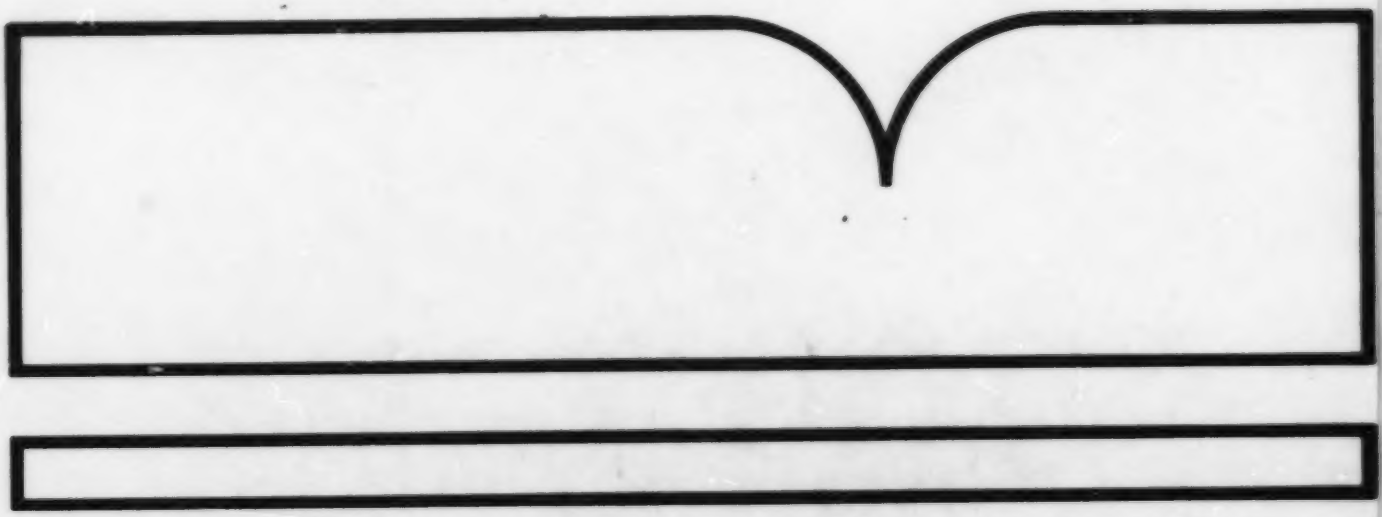

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Chromosomes from consecutive cluture passages of a developing cell line from fin fibroblasts of a male sheepshead (Archosargus probatocephalus) were analyzed. It was demonstrated that the modal chromosome number is 48. The chromosome types found in these cells included 8 submetacentrics, 8-12 acrocentrics, and 28-32 telocentrics. Evidence suggests that the primary type of chromosomal alteration that occurred in clutured SHR-1 cells is aneuploidy; a large metacentric chromosome of undetermined origin was observed.

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Analysis of a marine fish cell line from a male sheepshead

PAULA E. GREGORY, PATRICIA N. HOWARD-PEEBLES, RUDOLPH D. ELLENDER, AND BILLY J. MARTIN

DUE TO MANY PROBLEMS in methodology and analysis, progress in fish cytogenetics has not kept pace with that achieved in the cytogenetic studies of other vertebrate species. Cultured fish cell lines have helped in overcoming many of these mechanical problems but unfortunately variations in chromosome number and centric fusion frequently occurs in fish tissues cultured in vitro[6]. Thus, it is of interest to study the karyotype and any chromosomal alterations that occur in a newly initiated fish cell culture as it evolves toward established cell status.

The sheepshead (SHF-1) cell culture is a newly established marine fish cell line[4]. The cells exhibited fibroblast morphology and grew best at 26°C. The primary cells had $2n = 48$, but aneuploid changes had appeared in the culture by passage 11. In the present report, consecutive culture passages of a developing cell line from fin fibroblasts of a male sheepshead (*Archosargus probatocephalus*) were prepared for chromosome analysis. Chromosome counts and karyotypes were done on cells from each passage. The count data were analyzed for mean, mode, standard deviation, and confidence limits. Chromosome types were determined from the karyotypes.

Materials and Methods

Medium and culture techniques for the SHF-1 cells have been previously described[4]. In the present study, two consecutive sets of culture passages were examined: pass 13 through 32, pass 47 through 67.

Chromosome preparations were made by a modification of the method of Sumner et al.[7]. Colcemid was added to the cultures to a final concentration of approximately 0.25 μg/ml for 2–3 hours. Later, the medium was removed and the flask rinsed with trypsin-versene (ATV)[5]. The ATV treatment was repeated until all the cells were removed. The pooled cell suspension (original medium and all rinses) was centrifuged at 1000 rpm for 10 minutes; the supernatant was removed by aspiration. The cellular pellet was resuspended and treated for 20 minutes at room temperature with a hypotonic solution of 0.075 *M* KCl. Fixative (3 parts absolute methanol:1 part glacial acetic acid) was added (about ¼ ml) and the cells again collected by centrifugation. Thereafter, the supernatant was removed and 2 ml of fixative was carefully added to the cells. These cells were fixed for a minimum of 30 minutes at 4°C. The cells were washed in the fixative and centrifuged. After

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repeating this process three times, the cell suspension was dropped on clean, dry slides and air-dried. Slides were stained within 7–10 days in a standard Giemsa stain solution consisting of 1 ml R66 Giemsa (Gurr): 15 ml Gurr buffer (pH 6.8). They were rinsed, air-dried, and coverslipped.

The chromosome counts were made directly from the negative projections. Selected negatives were printed and karyotyped. Centromeric positions was determined by visual observation of the karyotypes.

Results

The karyotyped cells contained 8 submetacentric chromosomes (one large pair and three smaller pairs), 8–12 relatively large acrocentric chromosomes, and 28–32 telocentric chromosomes that range in size from fairly large to the smallest pair observed. Figure 1 shows a representative karyotype. For convenience, the chromosomes that could be easily identified by their morphology were grouped according to relative size and centromeric position.

Based on morphology, it was difficult to distinguish between some of the pairs of telocentric chromosomes. Often, it was difficult to differentiate between acrocentric chromosomes with minute short arms, and some telocentric chromosomes.

Analysis of the chromosome count data at each passage is shown in Table I. The statistical analyses indicated that some variation in the chromosome counts occurred; part of this variation was probably due to artefact caused by slide preparation. Most cells counted had $2n = 48 \pm 1$ and the overall modal chromosome number for all the passages studied was 48. These data correlate with those observed in the original

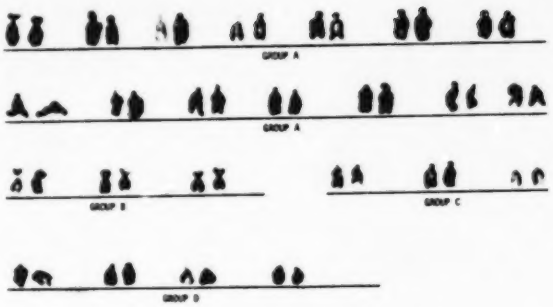

FIGURE 1—Karyotype of a SHF-1 cell having 48 chromosomes (passage 22).

passages[4] but appear to be more consistently distributed around the 48-chromosome count.

Figure 2 illustrates the morphology of a large metacentric chromosome that was observed in several consecutive passages. This metacentric chromosome was observed more frequently and more consistently in the higher passages. Analysis of cells containing this chromosome for arm number counts showed no relationship between the number of acrocentric and telocentric chromosomes and the presence of the large metacentric chromosome, i.e., there was no consistent loss of two acrocentric or telocentric chromosomes when the large metacentric was present.

Multiple attempts were made to produce GTG-bands in

Table I. Analysis of chromosome counts from each passage

Passage no.	Total spreads counted	Mean	s_t*	Confidence limits L_1	Confidence limits L_2	Mode
13	20	47	0.389	45.89	48.12	48
14	20	42	0.647	26.33	61.29	48
15	23	47	0.155	42.70	51.49	48
16	3	46	0.226	36.76	56.26	—
17	2	48	0.051	42.87	48.77	—
18	30	46	0.188	40.93	50.72	48
21	19	45	0.342	35.90	55.12	—
22	23	48	0.078	45.80	50.29	47
23	18	44	0.307	35.82	52.92	47
24	23	47	0.126	43.43	50.55	48
25	22	46	0.178	41.10	51.11	48
27	13	45	0.385	34.55	56.88	46
30	21	43	0.651	26.99	62.47	42
31	1	46**				
32	1	47**				
47	4	47	0.109	42.87	51.15	48
51	21	47	0.125	43.43	50.55	48
51(2)	18	47	0.112	43.75	50.19	48
52	22	47	0.109	43.85	50.08	48
55	21	45	0.371	35.28	55.98	47
56	20	48	0.117	44.57	51.32	48
57	20	47	0.141	42.98	51.04	47
59	25	48	0.107	44.87	50.98	48
60	22	48	0.153	43.59	52.37	48
62	4	43	0.351	31.09	56.61	47
66	4	45	0.467	29.29	64.13	—
67	23	49	0.448	36.89	62.82	48

* $\sqrt{\ }$ transformed standard deviation
** insufficient sample for statistical analysis

FIGURE 2—Karyotype of a SHF-1 cell having 47 chromosomes (passage 62). Arrow indicates the metacentric chromosome.

these preparations without success. The C-banding pattern has been reported[3].

Discussion

Many established vertebrate cell lines are characterized by a certain amount of chromosomal alteration from the primary tissue diploidy. In a well established marine fish fin fibroblast cell line from saltwater striped grunt[2], very little centric fusion was observed[6]. However, variations in chromosome number were found frequently. Thus, aneuploidy and sometimes even polyploidy could be expected in cultured fish tissue, especially in a younger cell line, i.e., prior to passage 75.

The occurrence of a single, large metacentric chromosome that was consistently observed through several passages, is highly suggestive of a centric fusion of two acrocentric or telocentric chromosomes. This metacentric chromosome could be maintained in the cell line through clonal evolution of the cells containing this fusion. Without G-banding, it is not possible to ascertain the origin of such a chromosome. Gene amplication[1] resulting in an increase in the repetitive DNA and a lengthening of the short arms of an acrocentric chromosome is another possible explanation for the origin of this chromosome. Unequal amounts of heterochromatin have been observed on either side of the centromere of this chromosome[3] but it is not clear if the larger C-banded area is truly a large heterochromatic or homogenous staining region (HSR) that is often associated with gene amplification. It is possible that such a chromosome could act as a marker for a single population of cells in this culture and subsequent studies should carefully monitor the cells for its presence. Any further discussion of the origin or meaning of this chromosome is at present entirely speculative.

It appears from the present study that the SHF-1 culture has a relatively stable karyotype and a modal chromosome number of 48. This karyotypic stability should allow SHF-1 cells to function as a model for in vitro studies of mutagens, clastogens, and chromosomal aberrations.

Summary

Chromosomes from consecutive culture passages of a developing cell line from fin fibroblasts of a male sheepshead (*Archosargus probatocephalus*) were analyzed. It was demonstrated that the modal chromosome number is 48. The chromosome types found in these cells included 8 submetacentrics, 8–12 acrocentrics, and 28–32 telocentrics. Evidence suggests that the primary type of chromosomal alteration that occurred in cultured SHF-1 cells is aneuploidy; a large metacentric chromosome of undetermined origin was observed.

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